

Cross-Sectional Serosurvey of Companion Animals Housed with SARS-CoV-2–Infected Owners, Italy

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We conducted a serologic survey among dogs and cats in Italy to detect antibodies against severe acute respiratory syndrome virus 2 (SARS-CoV-2). We found that SARS-CoV-2 seroprevalence was higher among cats (16.2%) than dogs (2.3%). In addition, seroprevalence was higher among animals living in close contact with SARS-CoV-2–positive owners.

After emerging in Wuhan, China, in December 2019, coronavirus disease (COVID-19), caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), rapidly became a serious threat to human health worldwide (1–3). Italy has experienced one of the highest rates of human deaths in the world (4).

Questions concerning the role of companion animals in the COVID-19 pandemic arose after a dog in Hong Kong reportedly tested positive for SARS-CoV-2 (5). In addition, the World Organisation for Animal Health defined COVID-19 as an emerging disease in animals and began promoting surveys on the prevalence of SARS-CoV-2 infections among animals (6). In this context, serologic tests are essential for rapid and accurate screening of animal populations.

Few studies have been conducted to clarify the effects domestic animals have in sustaining the SARS-CoV-2 transmission cycle (5,7–9; Q. Zhang et al., unpub. data, <https://www.biorxiv.org/content/10.1101/2020.04.01.021196v1>). Because Italy suffered high

COVID-19 incidence rates and the country has >32 million companion animals, health authorities were interested in examining virus transmission between humans and animals. We conducted a cross-sectional serologic survey among domestic dogs and cats in Italy to identify a possible association between SARS-CoV-2 infection in humans and animals. We used serologic tests to detect specific antibodies from animals living in close contact with SARS-CoV-2–positive human patients.

The Study

Blood was collected from pets during routine activities performed by veterinary practitioners, who shared serum samples with us. Owners provided written consent for research purposes. We used 198 samples, 130 from dogs and 68 from cats, collected during the March–June 2020 COVID-19 epidemic in Italy and 100 serum samples, 65 from dogs and from 35 cats, collected in different regions of Italy before 2019 as prepandemic controls.

A recombinant antigen corresponding to the nucleocapsid (N) protein of SARS-CoV-2 has been expressed in human embryonic kidney 293T cells, which have been used to develop Eradikit COVID19-IgG (IN3diagnostic, <https://www.in3diagnostic.com>) a sensitive and specific ELISA to detect SARS-CoV-2 antibodies in human serum samples. However, our initial attempts to validate the specificity of this ELISA on pet serum samples were unsuccessful. We switched the reaction from solid-phase to solution-phase kinetics using the same antigen and the specificity improved. Thus, we used a novel immunoassay, xMAP (Luminex Corp., <https://www.luminexcorp.com>), which is based on paramagnetic beads. We developed a flow cytometry-based system and applied it to serum samples from cats and dogs.

To define the test's specificity, we analyzed prepandemic samples and expressed results as the mean

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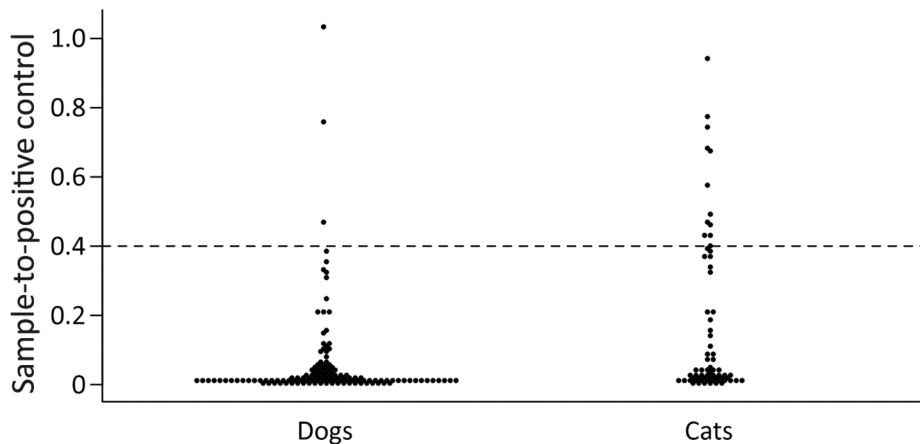


Figure 1. Distribution of sample-to-positive severe acute respiratory syndrome coronavirus 2 serology results among dogs and cats, Italy, March–June 2020. Horizontal dashed line represents the positive-negative discriminatory cutoff.

fluorescence intensity (MFI) ratio of a sample-to-positive control. On the basis of reactivity distribution, we set the discriminative cutoff to 40% MFI of the positive control. Using these specifications, we recorded diagnostic specificities of 96.5% (95% CI 87.9%–99.6%) for dog serum and 100.0% (95% CI 90.0%–100.0%) for cat serum.

Our choice of the viral N protein might raise concern because dogs and cats are susceptible to species-specific coronaviruses. The amino acid similarity between SARS-CoV-2 and the canine betacoronavirus, canine respiratory coronavirus, is slightly higher than canine and feline alphacoronaviruses (10), which

could explain the suboptimal specificity obtained in pre-epidemic dog samples. In fact, 2 serum samples gave reactivity slightly over the cutoff value. However, when potential cross-reactivity of the N protein between SARS-CoV-2 and endemic human coronaviruses was evaluated, no reactivity was shown against human coronaviruses 229E, OC43, HKU1, or NL63 by western blot or ELISA (11), suggesting that similar results might be expected from phylogenetically related feline and canine coronaviruses (12).

Among samples collected during the epidemic period, 7.1% (14/198) tested positive by the serologic test. In all, 147 animals (54 cats and 93 dogs) lived in households with SARS-CoV-2-positive owners. All 14 seropositive animals lived with SARS-CoV-2-infected owners and percent positivity was greater among cats than dogs (Table 1; Figure 1). Among animals living with SARS-CoV-2-infected owners, 20.4% (11/54) of cats and 3.2% (3/93) of dogs were seropositive.

Exact logistic regression analysis indicated a positive association between owners' infections and seropositivity in individual animals, after adjusting for animal species (Figure 2). The odds of finding ≥ 1 seropositive animal in a household were positively associated with owners' infection and with an increasing number of tested cats (Table 2; Appendix, <https://wwwnc.cdc.gov/EID/article/27/7/20-3314-App1.pdf>). The association with owner infection was only statistically significant based on a 1-tailed hypothesis, whether the outcome was measured at the animal or household level.

Using exact logistic regression, we noted the percent of positive results was greater for animals living indoors only than for animals with access to the outside (odds ratio 3.4, 95% CI 0.71–35.9), but the association was not statistically significant ($p = 0.15$). Because information on living conditions was missing

Table 1. Seropositivity among cats and dogs tested for antibodies against severe acute respiratory syndrome coronavirus 2, Italy, March–June 2020

Level of analysis	% Positivity among pets
Individual animal	
Owners' status*	
Infected, n = 147	9.5
Not tested, n = 49	0
Animal species	
Cat, n = 68	16.2
Dog, n = 130	2.3
Living condition†	
Indoor, n = 87	12.6
Outdoor, n = 51	3.9
Households tested, n = 156‡	
Owners' status	
Infected, n = 111	10.8
Not tested, n = 45	0
Cats tested in the household	
Yes, n = 51	19.6
No, n = 105	1.9
Dogs tested in the household	
Yes, n = 114	2.6
No, n = 42	21.4

*Information on owners' status was missing from 1 cat and 1 dog. Infected means positive molecular tests for the detection of SARS-CoV-2 in ≥ 1 owner in a household. Percent positivity at the household level was based on finding ≥ 1 seropositive animal.

†Information on pets' living conditions was missing for 43 dogs and 17 cats.

‡Households were considered positive if ≥ 1 pet was serologically positive. Households were divided into those in which ≥ 1 cat was tested and those in which ≥ 1 dog was tested.

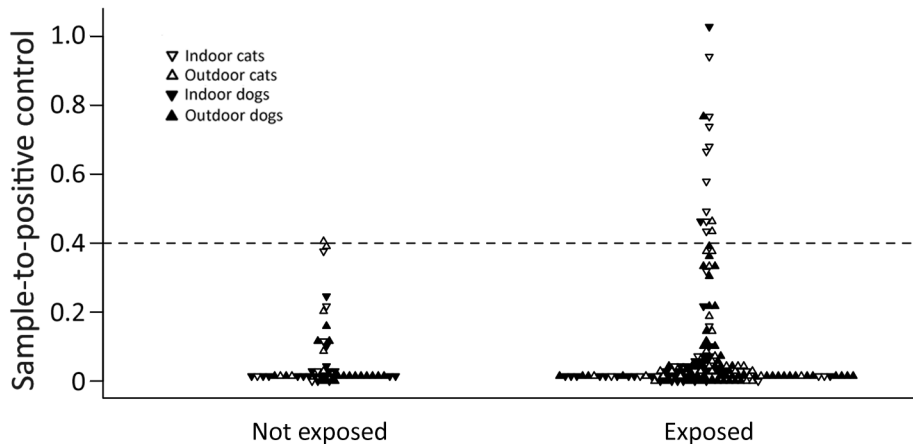


Figure 2. Distribution of sample-to-positive severe acute respiratory syndrome coronavirus 2 serology results among dogs and cats exposed and unexposed to positive owners, Italy, March–June 2020. Horizontal dashed line represents the positive-negative discriminatory cutoff.

for 60 animals, we did not include this factor in the exact logistic regression analysis (Tables 1, 2).

We found the proportion of serologic positivity increased with increasing length of exposure. We recorded the first SARS-CoV-2-positive animals 10 days after owners' diagnoses and all 14 seropositive cases were classified as positive after ≥ 54 days of exposure (Appendix).

Among 5/14 positive animals, owners reported that their pets experienced clinical signs concurrent with the owner's COVID-19 illness. In particular, a 10-year-old male dog showed respiratory signs (cough, sneezing) after which he had vomiting and diarrhea in concomitance with the onset of the owners' symptoms; a 1-year-old dog showed mild respiratory signs characterized by cough and sneezing; a 12-year-old female cat showed respiratory signs characterized by rhinitis with abundant nasal discharge. Furthermore, a 13-year-old female cat was hospitalized for a brachial cephalic thrombosis and a 3-year-old male cat was hospitalized for interstitial pneumonia. Of note, 3 asymptomatic SARS-CoV-2-positive cats belonged to a single-family cluster in which both owners tested positive and hospitalized.

Conclusions

We detected antibodies against the SARS-CoV-2 N protein in pets living with SARS-CoV-2-infected owners. A higher percentage of feline samples tested positive, confirming a higher susceptibility and prevalence in cats than in dogs reported in previous experiments (10,13). The susceptibility of cats to SARS-related human coronaviruses also was reported in 2003 when a study confirmed that cats were susceptible to infection and could transmit the virus to other in-contact animals (14). The association between seropositivity in animals and the confirmed SARS-CoV-2 infection in ≥ 1 of the animal's owners was statistically

significant ($p < 0.05$) based on a 1-sided test assuming the owner's infection could not reasonably exert a protective effect on pets' infection.

We could not draw conclusions concerning the direction of viral transmission in this cross-sectional study. Nevertheless, our results, coupled with the direction of the association between seropositivity and length of exposure to an infected owner and living indoors, suggest that the development of antibodies in pets might be a consequence of viral transmission from their owners. Additional studies with more statistical power could confirm these relationships.

Based on our results, future studies should focus on overcoming test limitations by improving specificity in dog serum samples through detailed epitope mapping of the N protein. Additional studies also should examine routes and risk factors for transmission of SARS-CoV-2 from infected persons to susceptible pets and the potential role of pets in the COVID-19 pandemic. Clinical and pathological consequences of SARS-CoV-2 infection in cats and dogs also warrant further research.

In conclusion, our study on companion animals housed with SARS-CoV-2-infected humans confirms the susceptibility of domestic cats under natural exposure. Our data statistically support other findings that cats are more susceptible than dogs

Table 2. Results of multivariable exact logistic regression of the association between severe acute respiratory syndrome coronavirus 2 seropositivity among cats and dogs and infected owners, Italy, March–June 2020

Level of analysis	Odds ratio (95% CI)	p value*
Individual animal		
Owners' status	6.1† (0.97– ∞)	0.055
Cat vs. dog	7.6 (1.9–44.4)	0.002
Household		
Owners' status	5.8† (0.9– ∞)	0.068
No. tested cats	2.5 (1.3–5.2)	0.008

*p value based on 2-tailed test.

†Median unbiased estimate.

and that living in contact with ≥ 1 SARS-CoV-2-infected person increases the risk for infection in pets. These results justify the need to adopt control measures in SARS-CoV-2-infected pet owners to reduce viral transmission to their companion animals.

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The study was carried out in compliance with the national legislation with authorization by the Ministry of Health Legislative Decree 26/2014 (authorization no. 694/2020-PR). Blood samples were collected during routine activities performed by veterinary practitioners. Written consent was obtained from all owners for research purposes.

About the Author

Dr. Colitti holds a research grant position in the Department of Veterinary Sciences, University of Turin, Italy. Her primary research interest is the diagnosis of animal infectious diseases.

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Appendix

Sampled Animals

The pre-epidemic panel consisted of serum samples collected from 65 dogs and 35 cats in different regions of Italy before 2019. During the March–June 2020 coronavirus disease (COVID-19) epidemic in Italy, serum samples of 198 animals from different regions of Italy were collected, including samples from 68 cats and 130 dogs. Among them, 54 cats and 93 dogs had been housed in close contact with COVID-19–affected patients whose infections were confirmed by detection methods for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). The remaining serum samples were from animals with no exposure or no confirmed exposure to SARS-CoV-2, although samples were collected in areas with confirmed viral circulation.

Written consent and the answers to a questionnaire were obtained from all pet owners, at the time of blood collection from animals. Owners were asked whether they had been tested for SARS-CoV-2 by nucleic acid tests and whether the test was positive. The following information was collected for each animal: name, sex, age, lifestyle (whether indoor or outdoor), and presence of chronic diseases. Time and duration of exposure to the infected owners were recorded for each animal, corresponding to the time between the first positive swab test in the owner and the end of possible animal exposure, such as when the owner was hospitalized or had a negative swab test. To avoid unnecessary health risks for veterinary practitioners, all samples were collected after the owners quarantined and had a negative SARS-CoV-2 test. Moreover, because animal serum samples were collected after their owners' quarantine was over, no animals were expected to be symptomatic. Only information about animal clinical signs that occurred concurrently with owners' illness were collected.

Serology

SARS-CoV-2 xMAP Assay

To set up the xMAP (Luminex Corp., <https://www.luminexcorp.com>) SARS-CoV-2 nucleocapsid (N) bead-based immunoassay, IN3diagnostic (<https://www.in3diagnostic.com>) provided a SARS-CoV-2 recombinant N. Paramagnetic Bio-Plex Pro COOH Beads (Bio-Rad, <http://www.bio-rad.com>) were coupled by carbodiimide chemistry with bovine beta-casein previously modified with Sulfo-NHS-LC-Biotin (Thermo Fisher Scientific, <https://www.thermofisher.com>). Then, recombinant N expressed in fusion with monomeric streptavidin was incubated with beads for 1 hour under agitation. After 2 washes, beads were resuspended in phosphate-buffered saline (PBS) and 0.05% sodium azide (NaN₃) and stored at 4°C protected from light until use.

Serum samples were diluted 1:200 in 100 µL of assay buffer (PBS, 0.05% Tween 20, and 0.05% NaN₃), in 96-well flat-bottom plates, mixed with 1,750 beads/well and incubated for 1 h at room temperature on a plate shaker set to 850 rpm, protected from light. The plate was washed twice by using a magnetic plate separator. As a secondary antibody, protein G (for dog serum) or protein A (for cat serum) was conjugated with phycoerythrin (PG-PE for dogs or PA-PE for cats; Moss Bio, <https://mossbio.com>) according to manufacturer's instructions. The resulting conjugate was used at 1:500 for dog serum and 1:250 for cat serum in assay buffer. Plates were incubated on a plate shaker for 30 min at room temperature, protected from light and then washed twice by using magnetic plate separator, as mentioned. Finally, 100 µL of wash buffer was added to each well and plates were read with the BioPlex200 (Bio-Rad) platform.

The median fluorescence intensity (MFI) was recorded for each sample and for negative and positive controls. Positive controls were represented by a hyperimmune serum produced in a goat immunized with the recombinant N antigen provided by the manufacturer in the Eradikit COVID19-IgG (IN3diagnostic) ELISA and was used for normalizing interplate experiments.

Statistical Analysis

We tested pre-epidemic serum samples and used these reactivities to assess the assay cutoff. We calculated the mean reactivity plus 3× the SD value and compared this with the reactivity of the positive control.

We performed statistical analysis to evaluate the role of different epidemiologic conditions in increasing the risk for positive results in pets. In detail, we evaluated the length of animal exposure to SARS-CoV-2–infected human patients, the animal species, the living habits, and the first day of exposure.

We tested the association between owner's confirmed infection and the odds of positive serologic test in animals by using the exlogistic command in Stata version 15.1 to perform exact logistic regression (1,2). This approach was justified by a quasicomplete separation of our results due to the frequency of 0 positive results among unexposed animals that belonged to untested owners. At the individual animal level, we adjusted for species, by including a dichotomous predictor, comparing cats versus dogs as the reference species.

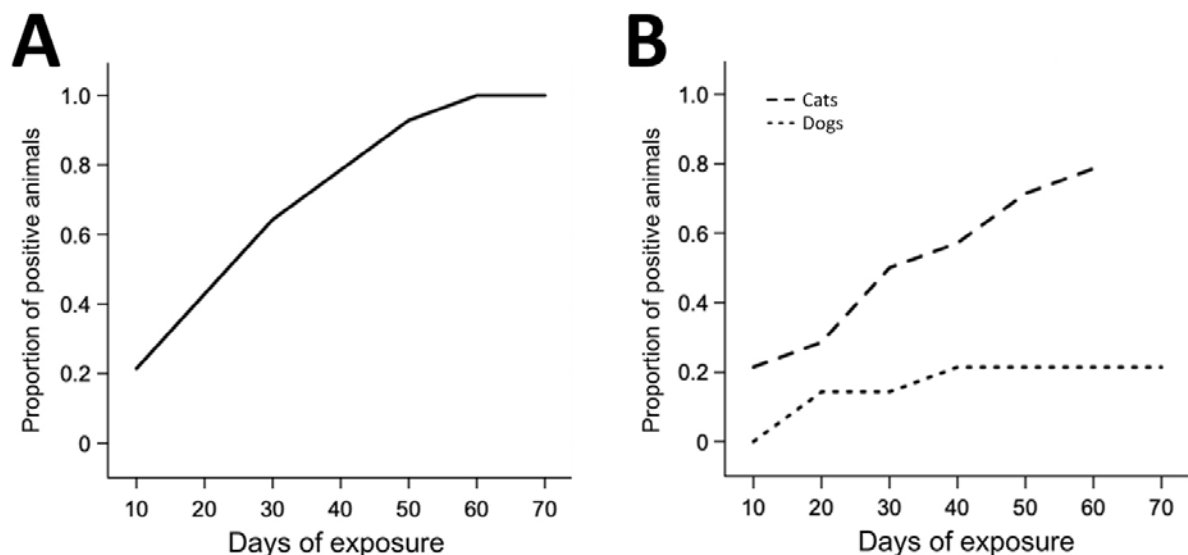
Animals from 156 households were tested. Due to the heterogeneous and generally small number of animals tested in each household, we did not use methods to account for nonindependence of test results on animals belonging to the same household, such as generalized estimating equations or random intercept logistic regression (3). As an alternative, we analyzed data at the household level. Accordingly, we considered a household positive if ≥ 1 tested animal was positive to antibodies against SARS-CoV-2; otherwise, the household was considered negative. Only 1 animal was tested in 8/12 (66.7%) positive households; 3 animals tested positive in 1 household; and ≤ 5 animals were tested in another household, and 1 tested positive. Among 144 negative households, only 1 animal was tested in 120 (83.3%); ≤ 4 animals were tested in 2 households. We analyzed the association between the owners' infection status and animals' serologic results at the household level by exact logistic regression (1) after adjusting for the number of tested animals, and for animal species, by including the number of tested cats and tested dogs as 2 separate predictors. We then eliminated the number of dogs, as a predictor, from the final model, since it was not associated with the outcome and its exclusion did not affect parameters for the other predictors. Pets' living conditions, indoors or outdoors, were not included in the exact logistic regression analysis with other predictors because information on this risk factor was missing for 60 animals.

Finally, we evaluated the time of exposure of each animal by considering the contact period between the animal and its owner when the SARS-CoV-2–positive owner was considered potentially infectious. We assumed the exposure period started on the date of the owner's first positive molecular diagnostic result and it ended when molecular diagnostic tests were negative or the owner left the house for hospitalization. Cumulative distribution of

positive animal samples was evaluated by dividing the exposure time in bins of 10-day units and used as categorical values for the description of serologic responses of tested animals.

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Appendix Figure. Cumulative distribution of SARS-CoV-2–positive serum samples among tested animals over time, Italy. The proportion of positive animals is correlated with the length of time animals were exposed to owners who tested positive for SARS-CoV-2. A) Cumulative positive results among all animals tested. B) Cumulative distribution among cat samples and dog samples. SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.